UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/905,212	07/13/2001	Venkatraman Ramakrishnan	26505-511	3863

30623 7590 01/13/2006

MINTZ, LEVIN, COHN, FERRIS, GLOVSKY
AND POPEO, P.C.
ONE FINANCIAL CENTER
BOSTON, MA 02111

EXAMINER	
TALAVERA, MIGUEL A	
ART UNIT	**PAPER NUMBER**
1656	

DATE MAILED: 01/13/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/905,212	RAMAKRISHNAN ET AL.	
	Examiner	Art Unit	
	Miguel A. Talavera	1656	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *October 19, 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-4,12 and 13* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-4, 12 and 13* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Application Status

1. A request for continued examination under 37 C.F.R. § 1.114, including the fee set forth in 37 C.F.R. § 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 C.F.R. § 1.114, and the fee set forth in 37 C.F.R. § 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 C.F.R. § 1.114. Applicant's submission filed on October 19, 2005 has been entered.

2. In response to the previous Office action, a Final rejection (mailed on November 17, 2004) and the subsequent Advisory Action (mailed on June 7, 2005), Applicants filed a response and amendment received on October 19, 2005. Said amendment cancelled claims 5-11 and 14-22 and amended claim 12. Applicants' arguments filed in said response have been fully considered but they are not deemed to be persuasive. Rejections and/or objections not reiterated from the previous Final rejection are hereby withdrawn. The following rejections and/or objections are either reiterated or newly applied to the instant application.

Priority

3. As previously noted, this Office action considers prior art before the earliest effective filing date of July 14, 2000.

Information Disclosure Statement

4. As previously noted, the information disclosure statements provided by Applicants (filed on February 12, 2002 and June 14, 2002) have been considered.

NEW-Objections to the Specification

5. The specification is objected to because the title is not descriptive. A new title is required that is clearly indicative of the invention to which the elected claims are drawn (see M.P.E.P. § 606.01). The Examiner suggests the following new title:

---Crystals of a *Thermus thermophilus* 30S ribosomal subunit bound to various antibiotics---

6. In the specification, the Abstract is objected to for not completely describing the disclosed subject matter (see M.P.E.P. § 608.01(b)). It is noted that in many databases and in foreign countries, the Abstract is crucial in defining the disclosed subject matter, thus, its completeness is essential. The Examiner suggests the following:

a. Replacement of the recitation "30S ribosome" in lines 3, 8 and 9 with ---30S ribosomal subunit---. In general, the art recognizes the existence of whole bacterial ribosomes having a sedimentation coefficient of 70S. In turn, whole bacterial ribosomes are composed of **two subunits**: **the small (30S) ribosomal subunit and the large (50S) ribosomal subunit** (Moore, P.B., *Biochemistry*, vol. 40, pp. 3243-3250, 2001).

WITHDRAWN-Basis for NonStatutory Double Patenting

7. Previous nonstatutory obviousness-type double patenting rejection with respect to claims 1-4, 7, 12, and 13, as recited in the previous Office action mailed February 24, 2004, is withdrawn. It would not have been obvious to use the unliganded crystals known in the art, exposed them to paromomycin and obtain the claimed crystals with the specified limitations in the instant application (see below for further details).

NEW-Claim Rejections - 35 U.S.C. § 112

The following is a quotation of the second paragraph of 35 U.S.C. § 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

8. Claim 12 is rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The phrase "having a resolution of at least about 3 Å" is unclear as to the limitations it imparts on the claimed subject matter or as to what said phrase encompasses. Because the recitation of the word **resolution** is not defined by the claims and the specification does not provide a standard for ascertaining the requisite degree for "at least **about** 3 Å", one of ordinary skill in the art would not be reasonably apprised of the scope of the claimed crystals.

The context of the word resolution is ambiguous. Said word could be use to describe either the quality of the **diffraction data** or the quality of the resulting **electron density map**. What quality features are shared among the claimed crystals? For example, are crystals with

diffraction limit of "at least about 3 Å" that lead to an **X-ray crystallographic electron density map** at a resolution of "at least about 3 Å" encompassed by the scope of the claim? Could crystals with a **diffraction limit** of "at least about 3 Å" leading to electron density maps of various resolutions be considered within the scope of the claim? For example, crystals from which data can be collected to 3 Å but by virtue of number of art-recognized factors (such as lack of completeness for the recorded reflections, lack of redundancy of the recorded reflections, overload of reflections, crystal mosaicity, crystal twinning, ice formation during data collection utilizing cryo-techniques, etc. [for a review on such factors see Weiss, J. Appl. Cryst., vol. 34, pages 130-135, 2001 and Gram, Acta Cryst.-D, vol. 55, pages 1641-1653, 1999]) the resolution of the generated electron density map can not be extended to the diffraction limit of the crystal.

Further, the word "about" in the phrase "at least **about** 3 Å" is a relative term that renders the claim indefinite. The word "about" is not defined by the claims, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. For example, are crystals with diffraction limit of at least 3.3 Å or at least 4 Å that lead to an electron density map extended to the diffraction limit of the crystal encompassed by the scope of the claim? What about crystals that **just diffract** to at least 3.3 Å or at least 4 Å and due to one or a combination of the above-identified factors, lead to an electron density map of less resolution than at least 3.3 Å or at least 4 Å? It is suggested that applicants clarify the meaning of the claim.

NEW-Written Description

The following is a quotation of the first paragraph of 35 U.S.C. § 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

9. Claims 1-4, 12 and 13 are rejected under 35 U.S.C. § 112, first paragraph, written description, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The instant claims are directed to all possible **crystals** containing a binary complex between a genus of **prokaryotic** 30S ribosomal subunits and the antibiotic paromomycin (species elected by Applicants in the response of March 31, 2003). The claimed genus includes **all naturally occurring** 30S ribosomal subunits from **all achaebacterial and eubacterial sources** as well as **all variants** from said sources that have been manipulated or reengineered for crystallization purposes.

To clarify the record, it is noted that the specification does not actually disclose any whole 30S ribosomal subunit as defined **by the small ribosomal components recognized in the art**:

> "In prokaryotes, the small (30S) subunit consists of a single RNA about 1500 nucleotides long (16S rRNA) and single copies of each about **20 different protein molecules** (3)." (see Moore, page 3243)"

As evidenced by Moore, said subunits consist of 20 different proteins, **namely S1-S20**, and the specification only discloses crystals of a 30S-like ribosomal subunit **lacking protein S1** (see specification, page 23, lines 30-31), which does not represent an art recognized, naturally occurring prokaryotic 30S ribosomal subunit.

While the specification describes isolation of a **30S-like** ribosomal subunit, crystal, space group, unit cell dimensions and X-ray crystallographic analysis leading to the structural coordinates a *Thermus thermophilus* 30S-like ribosomal subunit bound to paromomycin as shown in Tables 1A-1C, this disclosed species fails to represent a crystal of **any prokaryotic** 30S ribosomal subunit as encompassed by the claims.

The Court of Appeals for the Federal Circuit has held that a "written description of an invention involving a chemical genus, like a description of a chemical species, 'requires a precise definition, such as by structure, formula [or] chemical name,' of the claimed subject matter sufficient to distinguish it from other materials." UC California v. Eli Lilly, (43 USPQ2d 1398). For claims drawn to a genus, M.P.E.P. § 2163 states the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, *i.e.,* structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus. M.P.E.P. § 2163 states that a representative number of species means that the species which are adequately described are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus. At the time of the invention, it was well known in the art that the 30S ribosomal subunit consisted of **twenty** different proteins and 16S rRNA and that such ribosomal components comprising **prokaryotic** 30S ribosomal subunits were diverse in sequence. Such **heterogeneous chemical composition** of ribosomal components

across **prokaryotic** species leads to a diverse population of assembled **prokaryotic** 30S ribosomal subunits whose particular crystallization conditions are unique. Further, it was also well known in the art that the specific chemical composition of a macromolecule largely defines crystallization success and the corresponding macromolecular crystal of a ribonucleoprotein (RNP) complex, such as **prokaryotic** 30S ribosomal subunits. The general knowledge in the art, as exemplified by Hoggan *et al.* (Acta Crystallogr. D., vol. 59, pages 466-473, 2003), concerning crystallization of RNP complexes provide for a clear definition of the RNA and protein sequences as a parameter for macromolecular crystallizability:

> **"It is well known that a critical factor in obtaining suitable RNP crystals is the specific RNA construct or protein sequence variant used to make the RNP.** To crystallize a 58- nucleotide rRNA±L11 complex, an rRNA mutant was used that stabilizes the tertiary structure (Conn et al., 1998, 1999); the rRNA from another species also contains this stabilizing base triple (Wimberly et al., 1999). Crystallization of the polyA-binding protein-poly(A)$_8$ complex required screening five lengths of poly(A)$_n$ (Deo et al., 1999). Several constructs of the Sxl protein were screened and one was further mutated to obtain crystals of the tra mRNA complex (Handa et al., 1999). Diffraction-quality crystals of a U1A protein-snRNA complex were obtained only after 23 RNA constructs, 38 protein mutants and 90 unique complexes were prepared (Oubridge et al., 1995). The hepatitis δ virus (HDV; Ferré-D'Amaré & Doudna, 2000) and hairpin (Rupert & Ferré - D'Amaré, 2001) ribozymes were crystallized by first introducing the U1A protein-binding domain in order to prepare RNPs. Diffraction-quality crystals of a 104-nucleotide domain of 16S rRNA in complex with three ribosomal proteins were obtained only after biochemical characterization of several rRNA constructs (Agalarov et al., 2000). **These examples demonstrate that definition of a stable construct in both the protein and RNA is required to obtain RNP crystals, but that in addition to this, the introduction of single-residue variations can also be critical."**

These teachings on the sequence-dependence of crystallizability are also exemplified by the reference of Buts *et al.* (Acta Crystallogr. D., vol. 61, pages 1149-1159, 2005), regarding the impact of **natural variation** in a macromolecule on crystallization behavior:

> "Since the introduction of structural genomics, **the protein has been recognized as the most important variable in crystallization."** **"Five naturally occurring variants, differing in 1-18 amino acids, of the 177-residue** lectin domain of the F17G fimbrial adhesin were expressed and purified in identical ways. **For four out of the five variants crystals were obtained, mostly in non-isomorphous space groups, with diffraction limits ranging between 2.4 and 1.1 Å resolution."**

Although the sequence homology that exists between ribosomes, ribosomal subunits and components thereof from different prokaryotic species justifies the cross-species **functional** comparisons of ribosomes and the evolutionary analysis of said ribonucleoprotein complexes, the art, as exemplified by Mears *et al.* (J. Mol. Biol., vol. 321 pages 215-234, 2002), teaches that these general observations have been attributed to the **conserved sequence regions** (not full length sequence conservation or identity) forming ribosomal surfaces that drive the assembly of the ribonucleoprotein (i.e., intra-subunit protein-RNA interactions holding the ribosomal subunit or inter-subunit interactions between the 30S and 50S leading to formation of whole ribosomes) or that form the catalytic core, which actively participate in different aspects of protein synthesis (see Mears *et al.*, page 216):

> "Nucleotides that are 99–100% conserved are called "universally conserved residues", and these are represented as red spheres in Figure 1. While **23% of the 16 S rRNA positions are universally conserved** (Table 1), the region of greatest conservation in the small subunit surrounds the **area where the mRNA and tRNAs interact and move through the ribosome** (Figure 1(a)). **Other universally conserved areas are mostly at the interface where the small subunit interacts with the large subunit**, but the regions of domain I involved in the formation of intersubunit bridges have the lowest percentage of universally conserved residue"

However, such universal function of all prokaryotic 30S ribosomal subunits does not warrant the chemical identity (i.e. sequence identity) of the twenty different ribosomal proteins and the 16S rRNA comprising the 30S ribosomal subunit:

> "The alignment reveals a high sequence identity **(75%)** between *E.coli* and *T. thermophilus* 16S rRNA sequences."(see page 751 of Tung et al., Nat. Struct. Biol., vol. 9, pages750-755, 2002). "The sequence identities between the two sets of ribosomal sequences are **30-73%** (Table 1)." (see page 752 of Tung et al.)

Despite some sequence identity among components, Mears *et al* teaches that **non-identical sequences** among species can be mapped to areas that do not dictate ribosomal function, that is, the molecular surface of the ribosomal subunit:

> "The largest differences between widely diverged species **are at the periphery, away from the central core**."(see Mears *et al*, page 216)

The disclosed crystals comprising the *Thermus thermophilus* 30S-like ribosomal subunit with space group $P4_12_12$ (see specification, page 4, line 23) and unit cell parameters (see specification, Table on page 4 and page 12, lines 16-21) ***a***= 397.2-405.375 Å, ***b***= 397.2-405.375 Å and ***c***= 170.887-181.4 Å represent only one species of the claimed genus of **prokaryotic** 30S ribosomal subunit crystals.

Furthermore, it was well known in the art that a macromolecular crystal was defined by three repeating vectors a, b, and c, with **angles α, β, and γ, between them**. See pp. 586 and 2725 of the "Encyclopedia of Molecular Biology" (Creighton, T., John Wiley and Sons, Inc. New York, 1999). Thus, the instant specification is insufficient to put one skilled in the art in possession of the attributes and features of all crystals within the claimed genus.

There is no description of the identifying ribosomal components and the corresponding essential sequences for recognizing any crystal comprising any other prokaryotic 30S ribosomal subunit. There is a lack of written description (**angles α, β, and γ)** for the unit cell defining the crystal lattice. Moreover, there is no description of an actual reduction to practice or distinguishing identifying characteristics sufficient to show that the applicant was in possession of the claimed invention.

A description of what a material does (i.e., conserved structural and functional features of a prokaryotic 30S ribosomal subunit) rather than of what it is, usually does not suffice. The instant application does not more than describe the function of the 30S ribosomal subunit, which have a diverse chemical composition across prokaryotic species (in particular at the **molecular**

surface, which is often recognize in the art as one of the major driving forces of macromolecular crystallization [see maintained enablement rejection below]) and contains no information by which a person of ordinary skill in the art would understand that the inventors possessed the claimed crystals comprising **all prokaryotic 30S ribosomal subunits.** At best, it simply indicates that one should run tests on a wide spectrum of **prokaryotic 30S ribosomal subunits** in the hope that at least one of them will crystallize. Inadequate written description that merely identifies a plan to accomplish an intended result "is an attempt to preempt the future before it has arrived" *Fiers v. Revel*, 984 F.2d 1164,1171 9Fed.Cir. 1993).

Given the lack of description of a representative number of macromolecular crystals containing **any other prokaryotic** 30S ribosomal subunit and the unit cell parameters thereof, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicant was in possession of the claimed invention.

MANTAINED- Scope of Enablement

10. In view of the **lack of critical experimental details** (as described below in further detail) and that the disclosed crystal is not of a 30S ribosomal subunit but of a **30S-like** subunit (as argued above in the written description rejection), Claim 1-4, 12 and 13 rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make

and/or use the invention. In this case, the specification fails to enable a crystal of a prokaryotic 30S ribosomal subunit.

It is the examiner's position that undue experimentation would be required for a skilled artisan to make and/or use the claimed invention. Factors to be considered in determining whether undue experimentation is required are summarized in *In re Wands* (858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)) as follows: (A) The breadth of the claims; (B) The nature of the invention; (C) The state of the prior art; (D) The level of one of ordinary skill; (E) The level of predictability in the art; (F) The amount of direction provided by the inventor; (G) The existence of working examples; and (H) The quantity of experimentation needed to make or use the invention based on the content of the disclosure. See M.P.E.P. § 2164.01(a). The Factors most relevant to the instant rejection are addressed in detail below.

The breadth of the claims: Claims 1-4, 12 and 13 are so broad as to encompass all possible **crystals** containing a binary complex between the antibiotic paromomycin and the 30S ribosomal subunits from **any** prokaryotic source, such as those belonging to the archaebateria and eubacteria domains which are part of the **three-domain system**, a biological classification based on differences in **16S rRNA genes** introduced by Carl Woese in 1990 (see Woese et al. Proc. Natl. Acad. Sci. USA, vol. 87, pp. 4576-4579, 1990) that emphasizes the separation of prokaryotes into said two groups (domains). According to the interpretation provided above the claimed crystals include those comprising **all naturally occurring** 30S ribosomal subunits from the above-identified sources as well as **all variants** that have been manipulated or reengineered for crystallization purposes (i.e., rational design to enhance crystallizability of the RNP complex). The broad scope of claimed crystals is not commensurate with the enablement

provided by the disclosure with regard to the extremely **large number of prokaryotic 30S ribosomal subunits** and crystallization conditions, as wells as, the crystal characteristic based either on a collected diffraction pattern (i.e., space group and unit cell parameters; see claims 1-4 and presumably diffraction limit; see claim 12) or the final electron density map (i.e., presumably a map with the claimed limitation in resolution; see claim 12 and crystals defined by structure coordinates calculated from the map; see claim 13) as broadly encompassed by the instant claims. In this case the disclosure is limited to a crystal *Thermus thermophilus* 30S ribosomal subunit bound to paromomycin made by purifying 30S ribosomal subunits **lacking S1 ribosomal protein** and subjecting said RNP complex to crystallization by any suitable method known. Crystallization of said RNP complex was induced at 4 °C in a 0.1 M potassium cacodylate or 0.1 M MES buffered-solution at pH 6.5 containing 250 mM KCl, 75 mM NH_4Cl, 25 mM $MgCl_2$, 6mM 2-mercaptoethanol and 13-17% MPD. After 4-8 weeks of incubating said RNP under such conditions, the obtained crystals were incubated (soaked in) with paromomycin at concentration ranged "from about 10 to about 500 μM" (see specification, page 13, lines 10-25). Said crystals containing bound paromomycin have the space group $P4_12_12$ (see specification, page 4, line 23) and unit cell parameters (see specification, Table on page 4 and page 12, lines 16-21) ***a***= 397.2-405.375 Å, ***b***= 397.2-405.375 Å and ***c***= 170.887-181.4 Å and, although not explicitly stated, the Examiner presumes that at least native crystals (i.e. crystals without antibiotic) had a diffraction limit of 3.05 Å and that the **electron density map** derived from crystals containing antibiotic (of undisclosed diffraction limit) was extended to the resolution of the native dataset. To clarify the record, critical experimental details of the claimed crystals containing a binary complex between *T. thermophilus* 30S ribosomal subunit and the

antibiotic paromomycin **were not found** by the Examiner. For example, what is the **concentration of 30S ribosomal subunit** necessary for obtaining said crystals? What is the **diffraction limit of the crystals** from which the collected data was used to derived the structure coordinates shown in Table 1A-1C? Further, the art recognizes the **specific conditions** of a "crystal soak" as essential for obtaining diffraction-quality crystals. The reference of Skarzynski *et al.* (Acta Crystallogr. D., vol. 62, pages 102-107, 2006) teaches:

> "Soaking of crystals in appropriate solutions containing active compounds is the fastest way of creating protein-ligand complexes for suitable crystal systems, **but there are a number of issues related to this method**". "(iii) **Binding of potent compounds often causes conformational changes of the protein molecules and either complete or partial disruption of the crystal lattice. This effect can be very dependent on time and compound concentration. A number of initial trial soaking experiments are usually needed to establish conditions where compound binding is likely to occur without significantly deteriorating the diffraction properties of the crystal.**"

The nature of the invention: The invention is related to macromolecular crystals of prokaryotic 30S ribosomal subunit bound to various antibiotics. At the time of the invention, methods of macromolecular crystallization were well known in the art. However, the ability to crystallize a given macromolecule was, at the least, challenging to a skilled artisan as even minor alterations in the conditions of crystallization could result in altered crystal forms, crystals of sub-diffraction quality, or a lack of crystal growth. Specifically, this particular challenge was well documented in the art for crystals consisting of whole ribosomes and ribosomal subunits from prokaryotic sources (as described in further detail below).

The state of the prior art; The level of one of ordinary skill; and The level of predictability in the art: Regarding the claimed crystals, the state of the art at the time of the invention acknowledges a high level of unpredictability for making the full scope of claimed

crystals. It is well known in the art of macromolecular chemistry (herein **macromolecule** is meant to encompassed **folded/structured proteins, folded/structured nucleic acids and higher assembly complexes therefrom** containing associating protein subunits or associating protein and nucleic subunits) that crystallizing a macromolecule is a chancy and difficult process without any clear expectation of success. It is now evident that macromolecule crystallization is the main hurdle in macromolecular structure determination. For this reason, macromolecular crystallization has become a research subject in and of itself, and is not simply an extension of the structural biologist or crystallographer's laboratory. There are many references that describe the difficulties associated with growing macromolecular crystals. For example, Kierzek *et al.*, Biophys. Chem., vol. 91, pages 1-20, 2001, Wiencek, Annu. Rev. Biomed. Eng., vol. 1, pages 505-534, 1999 and Ke & Doudna, Methods, vol. 34, pp. 408-414, 2004.

> **"Crystallization is predictably the least predictable aspect of a structure determination project"** (see Ke & Doudna, page 408)
>
> **"The critical first step in X-ray crystal structure determination of biological macromolecules is to obtain suitable crystals. While crystallographic methods have advanced spectacularly in recent years with the advent of synchrotron-radiation sources, cryogenic techniques for data collection, area detectors and new procedures for phase determination and refinement, the problem of obtaining diffraction-quality crystals often remains a bottleneck. This is particularly true of complexes containing RNA, which are inherently more difficult to prepare, less stable and more conformationally variable. This challenge remains, even though the field of RNP structural biology has seen enormous recent progress with the structure determination of the ribosome and its subunits** (Yusupov *et al.*, 2001; Wimberly *et al.*, 2000; Ban *et al.*, 2000; Harms *et al.*, 2001)."(Hoggan *et al.* page 466)

It is commonly held that crystallization of macromolecules from solution is the major obstacle in the process of determining macromolecular structures. The reasons for this are many; **biological macromolecules are complex, and the delicate balance involving specific and**

non-specific interactions with other macromolecules and small molecules in solution, is difficult to predict.

Each biological macromolecule crystallizes under a unique set of conditions, which cannot be predicted in advance. Simply supersaturating the macromolecule to bring it out of solution may not work, the result would, in most cases, be an amorphous precipitate. Many precipitating agents are used, common ones are different salts, and polyethylene glycols, but others are known. In addition, additives such as metals and detergents can be added to modulate the behavior of the macromolecule in solution. Many kits are available (e.g. from Hampton Research ©), which attempt to cover as many parameters in crystallization space as possible, but in many cases these are just a starting point to optimize crystalline precipitates and crystals which are unsuitable for diffraction analysis. **Likewise, known crystallization conditions of another macromolecule with sequence identity or similar fold as the target macromolecule for crystallization are also often regarded as a starting point of parameters in crystallization space**. Successful crystallization is aided by knowledge of the macromolecules behavior in terms of solubility, dependence on metal ions for correct folding or activity, interactions with other molecules and any other information that is available. As evidence by Derewenda *et al.* (Acta Crystallogr. D., vol. 62, pages 116-124, 2006) the outcome of macromolecular crystallization is further compounded by the chemical composition of the macromolecule itself, in particular the **molecular surface area, and available surface sites** that might participate (i.e., crystal contacts) in holding together the three-dimensional array of macromolecules defining the crystal lattice:

> "Clearly, the protein's **microscopic surface properties** have a **critical impact on the thermodynamics and kinetics of crystallization.** It follows then that some proteins will crystallize more easily than others and that **the amino-acid composition and sequence are more informative with respect to possible crystallization outcome than is normally believed**."

Because macromolecular homologues, such as those ribosomal subunit from different prokaryotic species (see Mears *et al*, page 216 as argued above for lack of written description), differ primarily in their molecular surfaces and not their functional cores, a homologue from any given source is unpredictable to crystallize because one can not predict the particular juxtaposition of functional groups of neighboring molecules that would lead to crystal contacts.

> "It is arguable that homolog screening is a relic of the bygone era when it was the only means of diversifying the protein sample. In reality, it suffers from a major limitation: **the crystallizability of any given homolog is as unpredictable as that of the original target.**"

Because our understanding of crystallization mechanisms are still incomplete and the factors of macromolecular structure that are involved in crystallization are poorly understood, to make the macromolecular crystals encompassed by the scope of the genus of **prokaryotic 30S ribosomal subunit**, the following must be clear: the preparation and chemical composition of the molecules to be crystallized, and the crystallization conditions, including methods and reagents used. Crystallization experiments must be done in order to empirically determine if a macromolecule will crystallize, and preliminary X-ray diffraction experiments must be done in order to determine if the crystalline macromolecule will diffract to the resolution required for analysis. Therefore, precise instruction about how to make macromolecular crystals suitable for structure determination is required so that undue experimentation is not required.

Specifically, the art of ribosomal crystallography, as exemplified by Gluehmann *et al.* (Methods, vol. 25, pages 292-302, 2001), teaches how time consuming and difficult was to obtain diffraction quality crystals form a suitable **source** that would yield high resolution three-dimensional structures:

> "The cellular organelles translating the genetic code into proteins, the ribosomes, are large, asymmetric, flexible, and unstable ribonucleoprotein assemblies, **hence they are difficult to crystallize**. Despite **two decades of intensive effort and thorough searches for suitable sources**, so far **only three crystal types have yielded high-resolution structures**: two large subunits (from an archaean and from a mesophilic eubacterium) and **one thermophilic small subunit**."

The reference of Gluehmann *et al.* further states about the small ribosomal subunit:

> The **small ribosomal subunit is less stable than the large one**. We found that by exposing 70S ribosomes to a proteolytic mixture, the 50S subunits remained intact, **whereas the 30S subunits were completely** digested. Similarly, crystals obtained from 70S ribosomes assembled from purified subunits were found to consist only of 50S subunits (3), and the supernatant of the crystallization drop did not contain intact small subunits, but its proteins and fragmented 16S RNA chains. **Because of its lower stability, the small ribosomal subunit seemed to be hardly suitable for crystallographic studies. Indeed, contrary to the marked tendency of large subunits to crystallize, only one crystal form has so far been obtained from the small subunit (27, 28)."**

Along these same lines, the reference of Clemons et al. (J. Mol. Biol. vol. 310, pp 827-843, 2001, see IDS) teaches that the single reported crystal form of the *T.thermophilus* small ribosomal subunit was not reproducible and specific manipulation was done to enhance the reproducibility of crystals:

> "**The crystallization was not highly reproducible**. When the crystals were washed in mother liquor and then run on SDS/polyacrylamide and nondenaturing agarose/acrylamide gels, it was clear that they completely lack protein S1 (Figure 2(c)). **In subsequent experiments**, the S1-free population of 30 S subunits was selectively purified, either by hydrophobic interaction chromatography which resulted in a trailing edge to the main 30 S peak that was enriched in the S1-containing 30 S, or by preparative agarose/acrylamide gel electrophoresis. 54"

It is the Examiner position that such procedure may not have the same effect in all the target crystallization samples comprising **any prokaryotic** 30S ribosomal subunit. Screening for

suitable 30S ribosomal subunits forms any prokaryotic source would be constitute undue experimentation.

Further evidence regarding the **unpredictability** of obtaining crystals of a ribosomal subunit from a **different prokaryotic species** with an expectation that said crystals have the **same intrinsic parameters** such as **space group** (Claims 1-4, 12 and 13), **unit cell dimensions** (Claims 1-4, 12 and 13), **diffraction limit** (Claim 12), or **resolution** of resulting electron density map (claims 12 and 13) as those for a **known** crystal form and electron density map of a specific prokaryotic source. To specifically illustrate the unpredictability of the above-identified crystal parameters compounded with variation of a homologous samples, art recognized examples of crystal forms of the **universally conserved** large ribosomal subunit (50S) from two prokaryotic species, the archaebacteria *Haloarcula marismortui* (H50S) and the eubacteria *Deinococcus radiodurans* (D50S), will be set forth for the record of the instant Office action. The reference Ban, N., Freeborn, F., *et al.* (Cell, vol. 93, pp.11050-1115, 1998) teaches:

> "The crystals of *Haloarcula marismortui* large ribosomal subunits (HM 50S) used in this study were grown following procedures described by Yonath and coworkers (van Bohlen et al., 1991). **They are orthorhombic, space group $C222_1$, and with unit cell dimensions of: a= 210, b= 300, c= 570 Å. Although these crystals diffract to better than 3.5 Å resolution, the data used for this study were measured to 7.0 Å resolution** at Brookhaven National Laboratory (Table 1)"(see page1106).

A year later after publication of the above reference, the publication of Ban, N., Nissen, P., *et al.* (Nature, vol. 400, pp. 841-847, 1999) states:

> "The 5 Å -resolution map was obtained using multiple isomorphous replacement (MIR)/single wavelength anomalous difference (SAD) phasing together with intercrystal and non-crystallographic averaging **between three different crystal forms** (Table 1). **This was possible only after we discovered that the *H. marismortui* 50S subunit crystallizes both in an orthorhombic space group,**

> **$C222_1$, having unit-cell dimensions a= 212, b= 301 and c= 576 Å, and in a merohedrally twinned monoclinic space group, $P2_1$, whose cell dimensions and Laue symmetry are nearly indistinguishable from those of the orthorhombic form but whose diffraction intensities differ by about 30–35% (30 to 5.5 Å resolution data).**"(see page 843). "**As data extending to 3 Å resolution can be obtained from these crystals**, computation of a map at significantly higher resolution and its fitting by a complete atomic model of the 50S ribosomal subunit may soon be possible."(see page 846)

Moreover, the reference of Ban, N., Nissen, P., Hanssen, J. et al. (Science, vol. 289, pp. 905-920, 2000) teaches:

> "Several experimental approaches were used to extend the resolution of the electron density maps of the *H. marismortui* 50S ribosomal subunit from 5 to 2.4 Å. **A back-extraction procedure was developed for reproducibly growing crystals that are much thicker than those available earlier and that diffract to at least 2.2 Å resolution**"(see page 905)

Thus, for the **same species**, namely H50S, two crystal forms with different space groups and diffraction limits are known. However, crystals of a different **prokaryotic species**, the D50S, were obtained under different crystallization conditions that resulted in different space group, unit cell dimensions, diffraction limit and resolution of the electron density map. The reference Harms *et al.* (Cell, vol. 107, pp 679-688, 2001) discloses crystals of D50S having the space group I222 (see Table 1, page 680) and unit cell parameters (see Table 1, page 680) a= 170.8 Å, b= 409.4 Å and c= 695.6 Å, diffracting to a resolution of 3.0 Å and whose map resolution was extended to the diffraction limit of the crystal.

In view of these teachings, a skilled artisan would recognize that it is highly unpredictable as to whether diffraction-quality crystals of other **prokaryotic** 30S ribosomal subunits can be achieved using the crystallization parameters as set forth on p. 13 of the specification. Since **determination** of unit cell dimensions, space group, diffraction limit and resolution of electron density (map which allows for a three-dimensional model to be fit and the

subsequent calculation of structural coordinates [see claim 13]) **necessitates the diffraction data from a crystal,** a skilled artisan would recognize that it is highly unpredictable to know *a priori* if any other prokaryotic 30S ribosomal subunit would form crystals having the specified claimed limitations. That is, current macromolecular structure prediction is not accurate enough nor can macromolecule-solvent and macromolecule-macromolecule interactions be modeled with the necessary precision to pinpoint all contributions to the free energy of crystallization, *ab initio* crystallization prediction for macromolecules is not feasible (see Kierzek *et al.*).

The amount of direction provided by the inventor; The existence of working examples: The specification discloses only a single working example a crystal. It should be noted that this crystal is not a prokaryotic 30S ribosomal subunit because it lacks ribosomal protein S1, which is a component of naturally occurring 30S ribosomal subunits. It should also be noted that the specification fails to disclose critical experimental details that are necessary for a skilled artisan to reproduce the disclosed crystal. As noted previously, the concentration of 30S ribosomal subunit, specific conditions for "soaking-in" paromomycin (i.e., time of incubation), etc. Other than this working example, the specification fails to provide guidance for altering the crystallization conditions for crystallizing any other prokaryotic 30S ribosomal subunit with an expectation of obtaining diffraction-quality crystals or any other conditions with an expectation of obtaining diffraction-quality crystals.

The quantity of experimentation needed to make or use the invention based on the content of the disclosure: While methods of macromolecular crystallization were known at the time of the invention, these methods are specific to a particular macromolecule as evidenced by the above teachings. Thus, a skilled artisan is left to experiment by a trial and error process to

determine whether the disclosed crystallization conditions can be applied to crystallization of other prokaryotic 30S ribosomal subunits or whether said subunits can be crystallized at all.

In view of the overly broad scope of the claims, the lack of guidance and working examples provided in the specification, the high level of unpredictability as evidenced by the prior and current state of the art, and the amount of experimentation required to make all the crystals as broadly encompassed by the claims, undue experimentation would be necessary for a skilled artisan to make and use the entire scope of the claimed invention. Applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims. The scope of the claims must bear a reasonable correlation with the scope of enablement (*In re Fisher*, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Response to Arguments

11. The response filed on October 19, 2005 has been fully considered, however, is not persuasive. Note that new objections to the specification have been raised. Additionally, note that new grounds of rejections have been instituted under 35 U.S.C. § 112, second paragraph for the reasons stated above. Moreover, the rejections under 35 U.S.C. § 112 first paragraph (written description and enablement) have been maintained.

Written Description

Applicants argue that the rejection is overcome by amendment. However, this is not found persuasive for the reasons stated above.

Enablement

Applicants argues:

1) the specification provides sufficient guidance to make all prokaryotic 30S ribosomal subunits as encompassed by the claims.

2) the cited reference of Drenth does not reflect the state of the art at the time of the invention.

3) the structural conservation among prokaryotic ribosomal subunits is so great that a single working example enables a skilled artisan to crystallize other prokaryotic 30S ribosomal subunits without difficulty.

4) claims of an issued US patent are similar to claims of the instant application.

Regarding the above-identified items 1-3, applicants are reminded that the instant claims are drawn to a crystal and not to a conserved macromolecular structure or fold. As argued above, the structural conservation, functional conservation or sequence identity of a macromolecule does not warrant crystallizability. Making a crystal is an unpredictable undertaking largely influenced by the chemical and physical identity of the target macromolecule, as evidence by the references representing the general state of the art regarding macromolecular crystallization and the specific references to the subject matter of ribosomal crystallography. In this case, the specification fails to enable the claimed invention for the reasons stated above. Regarding item 4,

applicants are reminded that each application is independent and is examined on its own merits. For reasons stated above, the enablement rejection remains maintained.

Citation of Relevant Prior Art

12. The following are cited to complete the record:

Tocilj *et al.* (Proc. Natl. Acad. Sci. USA, vol. 96, 14252-14259, 1999, see IDS) teach crystals of *Thermus thermophilus* 30S ribosomal **without any bound antibiotic** belonging to the space group $P4_12_12$ and with diffraction limit of 3 Å. However crystals were reported to have unit cell dimensions of a= b= 407 Å , c = 176 Å, **which are not** unit cell dimensions within the scope of the claimed crystals. Even though binding of paromomycin to the 16S rRNA, the RNA component of the 30S ribosomal subunit, was known in the art (De Stasio *et al.* EMBO J., vol. 8, pp. 1213-1216, 1989) and there was clear motivation to explain the effect of a paromomycin induce a **conformation** of 16S rRNA in molecular terms and its correlation with ribosome function and various antibiotic resistance mechanisms (Pape *et al.* Nat. Struct. Biol., vol. 7, 104-107, 2000, see IDS), it would not have been obvious to use the crystals of Tocilj *et al.*, exposed them to paromomycin and obtained crystals with the specified claim limitations (regarding undue experimentation of soaking methods, see Skarzynski *et al.*)

Conclusion

13. Claims 1-4, 12 and 13are rejected for the reasons identified in the numbered sections of the Office action. Applicants must respond to the objections/rejections in each of the numbered sections in the Office action to be fully responsive in prosecution.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Miguel A. Talavera whose telephone number is (571)272-3354. The examiner can normally be reached on M-F, 8:30am-5:00pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Kathleen M. Kerr can be reached on (571)272-0931. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

DAVID J. STEADMAN, PH.D.
PRIMARY EXAMINER

Miguel A. Talavera, Ph.D.
December 21, 2005